Biology 5357: Chemistry & Physics of Biomolecules
Fall 2021

Lecture 1: Discovery of the Lipid Bilayer Cell Membrane and the Proteins Embedded Within

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Reading for this week:

Required reading:

https://dx.doi.org/10.1085/jgp.201812153

https://dx.doi.org/10.1126/science.175.4023.720

Other useful references:

The cell membrane
The biological barrier that enables life

E. coli

Erythrocyte - red blood cell

https://ccsb.scripps.edu/goodsell/art/
The first visualization of ‘cells’

Robert C. Hooke, *Micrographia: or Some Physiological Descriptions of Miniature Bodies Made by Magnifying Glasses with Observations and Inquiries thereupon* (1665)
### Biology requires boundaries

<table>
<thead>
<tr>
<th>Archaea</th>
<th>Phylum</th>
<th>Representative organisms</th>
<th>Representative micrograph</th>
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<tbody>
<tr>
<td>Euryarchaeota</td>
<td>Methanogens</td>
<td>Methane production causes flatulence in humans and other animals.</td>
<td><img src="https://www.khanacademy.org/science/biology/bacteria-archaea/prokaryote-metabolism-ecology/a/prokaryote-classification-and-diversity" alt="Halobacterium strain NRC-1" /></td>
</tr>
<tr>
<td>Crenarchaeota</td>
<td>Sulfolobus</td>
<td>Members of this genus grow in volcanic springs at 75°C and 80°C and a pH between 2 and 3.</td>
<td><img src="https://www.khanacademy.org/science/biology/bacteria-archaea/prokaryote-metabolism-ecology/a/prokaryote-classification-and-diversity" alt="Sulfolobus being infected by bacteriophage" /></td>
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<tr>
<td>Nanoarchaeota</td>
<td>Nanoarchaeum equitans</td>
<td>This species was isolated from the bottom of the Atlantic Ocean and from a hydrothermal vent at Yellowstone National Park. It is an obligate symbiont with Ignicoccus, another species of archaea.</td>
<td><img src="https://www.khanacademy.org/science/biology/bacteria-archaea/prokaryote-metabolism-ecology/a/prokaryote-classification-and-diversity" alt="Nanoarchaeum equitans" /></td>
</tr>
<tr>
<td>Korarchaeota</td>
<td>No members of this species have been cultivated.</td>
<td><img src="https://www.khanacademy.org/science/biology/bacteria-archaea/prokaryote-metabolism-ecology/a/prokaryote-classification-and-diversity" alt="This image shows a variety of korarchaeota species from the Obsidian Pool at Yellowstone National Park." /></td>
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</tbody>
</table>

- Prevent the passage of large charged molecules like proteins, DNA & RNA
- Prevent passage of small charged molecules like ATP and nutrients
- Hold ionic gradients
- Hold pH gradients
- Fluid and flexible
- Sustain diverse living conditions (temperature, acidity/alkalinity, pressure)

Not just plants - microbes and animal cells

Antonie van Leeuwenhoek observations of microbes (1677)

(a) Rotifers, hydra and vorticellids associated with a duckweed root, from a Delft canal.

From Leeuwenhoek [16]. (b) Bacteria from Leeuwenhoek’s mouth; the dotted line portrays movement. From Leeuwenhoek [17]. Copyright © The Royal Society.

(a) Replica of a single-lens microscope by Leeuwenhoek (Image by Jeroen Rouwkema. Licensed under CC BY-SA 3.0 via Wikimedia Commons). (b,d) Photomicrographs taken using simple single-lens microscopes including one of Leeuwenhoek’s originals in Utrecht, by Brian Ford (Copyright © Brian J. Ford). (b) An air-dried smear of Ford’s own blood through the original van Leeuwenhoek microscope at Utrecht, showing red blood cells and a granulocyte with its lobed nucleus (upper right; about 2 µm in diameter). (c) Spiral bacteria (Spirillum volutans) imaged through a replica microscope with a lens ground from spinel; each bacterial cell is about 20 µm in length. (d) The intestinal protozoan parasite Giardia intestinalis imaged through a replica soda-glass produced by Brian Ford [28,29].
Invisible boundaries must be flexible

August Johann Rösel von Rosenhof, who named his discovery

"Der Kleine Proteus" ("the Little Proteus") 1755
The first uttering of a cellular “membrane”

The development of cells according to Schleiden. This figure has been drawn for clarity from descriptions by Schleiden and Schwann, but these authors never tried to provide such a synthetic depiction in their work.

Schwann’s model was very similar, except for his opinion that new cells could also crystallize from cytoplasm outside previous cells. 

https://doi.org/10.1186/s13062-014-0032-7
Other theories of cell boundaries

Aggregation of the colloidal protoplasm

A. In this vision, the cell is devoid of any membrane and all the properties of the cell are defined by the activity of the protoplasmic colloid. B. The cell is surrounded by an external layer (membrane) of which the nature is distinct to the rest of the protoplasm. Yet, in this view, the inside of the cell remains a colloid.

Hardened shells in molecular gastronomy

Calcium chloride and sodium alginate combine to polymerize, forming a hardened aggregate layer around a volume of solution. Shown here are the olives from Ferran Adria’s restaurant elBulli.

Biological phase separation

Riback et al., Cell 2017; https://doi.org/10.1016/j.cell.2017.02.027
Studies of plasmolysis

Hypertonic

Isotonic

Hypotonic

Hypertonic

Isotonic

Hypotonic

H\(_2\)O

H\(_2\)O

H\(_2\)O
Studies of plasmolysis

- Hypertonic: Plasmolyzed
- Isotonic: Flaccid
- Hypotonic: Turgid

H₂O

Vacuole
The theory of osmotic pressure

$$\Pi = icRT$$

- $$\Pi$$ - osmotic pressure
- $$i$$ - van’t Hoff factor (dissociable species)
- $$c$$ - N/V - concentration
- $$R$$ - gas constant
- $$T$$ - temperature (K)
Overton’s return to plasmolysis

Plasmolysis reveals invisible barriers within living cells. (A and B) A normal Spirogyra cell (A) and the cell during plasmolysis (B). From Overton (1895), Fig. 1 is adapted from Vierteljahresschr. Naturforsch. Ges. Zürich.

Figure 1: The wide range of membrane permeabilities of different compounds in the cell. Membranes are more permeable to uncharged compounds and least permeable to charged ions. Note that the existence of ion channels will make the apparent permeability when they are open several orders of magnitude higher. The units are chosen as nm/s and several nm is the characteristic membrane width. Figure adapted from R. N. Robertson, The Lively Membranes, Cambridge University Press, 1983. The value for glucose is smaller than in Robertson based on several sources such as Handbook of Biochemistry and Metabolism, Cambridge University Press, 1970. Other sources: BNID 110830, 110807, 110729, 110731, 110816, 110824, 110806.

Cell membranes are made of lipids
An interesting property about olive oil

"...But recollecting what I had formerly read in Pliny, I resolved to make some experiment of the effect of oil on water, when I should have opportunity..."

...At length being at Clapham where there is, on the common, a large pond, which I observed to be one day very rough with the wind, I fetched out a cruet of oil, and dropt a little of it on the water. I saw it spread itself with surprising swiftness upon the surface; but the effect of smoothing the waves was not produced; for I had applied it first on the leeward side of the pond, where the waves were largest, and the wind drove my oil back upon the shore. I then went to the windward side, where they began to form; and there the oil, though not more than a teaspoonful, produced an instant calm over a space several yards square, which spread amazingly, and extending itself gradually till it reached the leeside, making all that quarter of the pond, perhaps half an acre, as smooth as a looking-glass.

After this, I contrived to take with me, whenever I went into the country, a little oil in the upper hollow joint of my bamboo cane, with which I might repeat the experiment as opportunity should offer; and I found it constantly to succeed.

In these experiments, one circumstance struck me with particular surprise. This was the sudden, wide, and forcible spreading of a drop of oil on the face of the water, which I do not know that anybody has hitherto considered. If a drop of oil is put on a polished marble table, or on a looking-glass that lies horizontally; the drop remains in its place spreading very little. But when put on water it spreads instantly many feet round, becoming so thin as to produce the prismatic colours, for a considerable space and beyond them so much thinner as to be invisible, except in its effect of smoothing waves at a much greater distance..."
The first studies of surface tension of oil monolayers

Surface Tension.

I shall be obliged if you can find space for the accompanying translation of an interesting letter which I have received from a German lady, who with very homely appliances has arrived at valuable results respecting the behaviour of contaminated water surfaces. The earlier part of Miss Pockels’ letter covers nearly the same ground as some of my own recent work, and in the main harmonizes with it. The later sections seem to me very suggestive, raising, if they do not fully answer, many important questions. I hope soon to find opportunity for repeating some of Miss Pockels’ experiments.

Kayleigh.
March 2.

Brunswick, January 10.

My Lord,—Will you kindly excuse my venturing to trouble you with a German letter on a scientific subject? Having heard of the fruitful researches carried on by you last year on the hitherto little understood properties of water surfaces, I thought it might interest you to know of my own observations on the subject. For various reasons I am not in a position to publish them in scientific periodicals, and I therefore adopt this means of communicating to you the most important of them.

First, I will describe a simple method, which I have employed for several years, for increasing or diminishing the surface of a liquid in any proportion, by which its purity may be altered at pleasure.

A rectangular tin trough, 70 cm. long, 5 cm. wide, 2 cm. high, is filled with water to the brim, and a strip of tin about 1 cm. wide laid across it perpendicular to its length, so that the under side of the strip is in contact with the surface of the water, and divides it into two halves. By shifting this partition to the right or the left, the surface on either side can be lengthened or shortened in any proportion, and the amount of the displacement may be read off on a scale held along the front of the trough.

No doubt this apparatus suffers, as I shall point out presently, from a certain imperfection, for the partition never completely shuts off the two separate surfaces from each other. If there is a great difference of tension between the two sides, a return current often breaks through between the partition and the edge of the trough (particularly at the time of shifting). The apparatus, however, answers for attaining any condition of tension which is at all possible, and in experiments with very clean surfaces there is little to be feared in the way of currents breaking through.
High resolution measurements of lipid monolayers

The Langmuir trough (Pockels)

https://itn-snul.net/2014/10/langmuir-trough/
Membranes as lipid bilayers

A

Erythrocytes

Lipid extraction

Cell lipid surface measurement

Ratio comparison

Total cell surface estimate

B

Gorter & Grendel 1925
2:1 ratio
Lipid bilayer

Dervichian & Macheboeuf 1938
1:1 ratio
Lipid monolayer

Bar et al. 1966
1.3:1 ratio
Lipid bilayer
Davson & Danielli’s paucimolecular model
Seeing is believing - the ultrastructure of membranes

http://doi.org/10.1085/jgp.52.1.257

Robertson 1964
The unit membrane hypothesis

Figure 4: Robertson’s now-infamous diagram of a hypothetical cell, illustrating, its legend said noncommittally, “relationships of the cell membrane to various cell organelles.” In the original text relating to this figure, Robertson spoke more boldly, proposing that “it would not be unreasonable to conceive of an intracellular ‘circulatory system’ directly connected to the outside world in some places.” Given what is now known about endo- and exocytosis, as well as what is known about membrane trafficking inside the cell, such an extreme degree of membrane continuity is considered unlikely; but Robertson’s basic point was that because all cellular membranes are constructed along the same architectural principles, they are at least potentially capable of becoming continuous with each other. (From: Sci. Amer., 206: 64-72, 1962)
Fluid lipid bilayers

Rapid intermixing of surface antigens

Frye & Edidin, 1970
A summary of membrane models over the past 200+ years

1850s

1925, 1935

1959

Anselmi et al., 2019
The problem of putting protein in membranes

Polypeptides are expected to be unstable in membranes due to unsatisfied charges in the backbone.

Davson & Danielli model

[Diagram of Davson & Danielli model showing lipoid molecule, protein molecule, and polar pore]
Freeze-fracture EM reveals integral membrane proteins

Moor and Mühlethaler, 1963
The fluid mosaic model

Fig. 1. A phospholipid bilayer: schematic cross-sectional view. The filled circles represent the ionic and polar head groups of the phospholipid molecules, which make contact with water; the wavy lines represent the fatty acid chains.

Fig. 3. The lipid-globular protein mosaic model with a lipid matrix (the fluid mosaic model): schematic three-dimensional and cross-sectional views. The solid bodies with slippled surfaces represent the globular integral proteins, which at long range are randomly distributed in the plane of the membrane. At short range, some may form specific aggregates, as shown. In cross section and in other details, the legend of Fig. 2 applies.

Fig. 5.2. Schematic illustration of the way lipids and proteins may be organized around (a) a curved membrane; and (b) at contacting sites.
A gold-mine of highly ordered membrane protein

- Stoeckenius & Rowen (1967) isolated purple membranes from H. Halobium archaea (> 4.3 M salt conc.)
- Osterhelt & Stoeckenius (1970) identified a single molecular species with MW 26 kDa formed the purple membrane. They showed that it was responsible for the color due to the binding of retinal, bound via a Schiff base linkage to lysine 216.
- First membrane protein structure in 1975 by Henderson & Unwin by EM diffraction

Deisenhofer, …, Huber & Michel (1985) solved the first x-ray crystal structure of a membrane protein, the photosynthetic reaction centre of Rhodopseudomonas viridis at 3 Å resolution

Detergent solubilization was critical

Figure 1. The reaction center from the photosynthetic bacterium *Rhodopseudomonas viridis*. The electron transfer cofactors are depicted as structures comprised of red spheres. The cytochromes supply electrons to a bacteriochlorophyll dimer called the "special pair" that absorbs light and reduces a bacteriopheophytin intermediate. There are four proteins, the "L" and "M" subunits (dark and light blue) whose alpha-helices span the membrane bilayer, the "H" subunit (yellow) on the side of the reaction center that accepts the electrons released by absorption of photons, and the cytochrome subunit that ligates the electron donor hemes (gold).
MEMBRANE PROTEINS

ALL PROTEINS

https://www.rcsb.org/stats/growth/growthreleased-structures
A summary of membrane models over the past 200+ years

1850s

1925, 1935

1959

1972

Anselmi et al., 2019
A molecular understanding of the lipid bilayer

http://lipidbuilder.epfl.ch/home